human cadaveric lungs with a synthetic medium and measuring levels of factor VIII and other proteins during the course of perfusion. Significant amounts of factor VIII (and von Willebrand factor) appeared in the perfusate while factor V, fibrinogen, and immunoglobulin G (IgG) remained unchanged for the duration of the experiments. In vitro studies were performed on conditioned medium from cultivation of fibroblasts, epithelial cells, macrophages, smooth muscle cells, and microvascular endothelial cells. Factor VIII synthesis was observed only in the endothelial cells. The rate of factor VIII synthesis by these endothelial cells in vitro could be extrapolated to account for about 20% of the human factor VIII produced in vivo. Thus, the pulmonary endothelium makes a significant contribution to the factor VIII found in the circulation.

This kind of study could be profitably applied to other organs, and it would not be surprising if factor VIII synthesis could be demonstrated in other nonhepatic sites. Perhaps more of this work will appear in the pages of *Blood*, where much of this story has already been told.

This work is the opinion of the author and does not constitute US government policy. ■

REFERENCES

1. Bontempo FA, Lewis JH, Gorenc TJ, et al. Liver transplantation in hemophilia A. Blood. 1987;69:1721-1724.
2. Webster WP, Zukoski CF, Hutchin P, et al. Plasma factor VIII synthesis and control as revealed by canine organ transplantation. Am J Physiol. 1971;220:1147-1154.
3. Do H, Healey JF, Waller EK, Lollar P. Expression of factor VIII by mouse liver sinusoidal endothelial cells. J Biol Chem. 1999;274:19587-19592.
4. Doering CB, Josephson CD, Craddock HN, Lollar P. Factor VIII expression in azoxymethane-induced murine fulminant hepatic failure. Blood. 2002;100:143-147.
5. Healey JF, Lubin IM, Lollar P. The cDNA and derived amino acid sequence of porcine factor VIII. Blood. 1996;88:4209-4214.
6. Cameron C, Notley C, Hoyle S, et al. The canine factor VIII cDNA and 5′ flanking sequence. Thromb Haemost. 1998;79:317-322.
7. Naylor JA, Green PM, Rizza CR, Giannelli F. Factor VIII gene explains all cases of haemophilia A. Lancet. 1992;340:1066-1067.

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Comment on Rudd et al, page 638

Chips, candidate genes, and CLL

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Rudd and colleagues have conducted a large-scale association study using nonsynonymous SNPs on a population sample of patients with CLL and healthy controls and identified a group of plausible candidate SNPs, including *ATM*, *CHEK2*, and *BRCA2*.

The search for the specific genes that account for susceptibility to most human disease in general and hematologic cancer in particular is a pivotal goal in human genetics and a major anticipated benefit of the sequencing of the human genome. The handful of major (ie, high-penetrance) genes identified for a few human cancers (eg, *BRCA*s in breast cancer) account for only a small component of the genetic predisposition that exists, and the minor (low-penetrance) genes that likely account for the balance of susceptibility are not known with certainty for any human malignancy, despite more than a decade of candidate testing. The situation with chronic lymphocytic leukemia (CLL) is typical. Striking kindreds with multiple patients with CLL, population registry, and twin studies clearly implicate heredity as important in CLL. Family history predicts risk of CLL far better than any known extrinsic risk factor. Yet to date, the genes that account for elevated risk in relatives of patients with CLL are unknown. Separate linkage studies using the largest existing collections of high-risk kindreds in the United States[1] and the United Kingdom[2] have failed to provide strong evidence for specific chromosomal regions that may harbor these genes. Linkage is a powerful technique, but among its limitations is the requirement for many families, and this approach cannot detect the multiple weaker genes (those that convey less than a 4-fold risk) that are precisely the ones thought to contribute most to hereditary susceptibility. Thus, so-called "association studies," based in the general population, are an essential complement to family studies. To date, however, population-based studies that test one gene or a few genes at a time have not proven fruitful. While critical observers have emphasized the need for attention to study design (need for adequate power, careful control selection, attention to population structure and false positives), there is an increasing consensus that new genomic technologies that rapidly assess multiple genomic variants will provide the breakthrough. Here, 2 general approaches are possible. The first, or indirect approach, uses chips with tens to hundreds of thousands of single nucleotide polymorphisms (SNPs) and tests for statistical associations of each SNP with disease. Since the SNP detected on the chip that exhibits the association may not be the one that accounts for the critical disease-causing alteration, but only one nearby (ie, in linkage disequilibrium), as in linkage analysis, a follow-up round of genetic studies must be conducted to verify the locus and find the specific gene. A second approach is the "direct" method selected by Rudd and colleagues in this issue of *Blood*. Using diverse genomic tools, they selected only SNPs calculated to cause an amino acid change (nonsynonymous SNPs [nsSNPs]), thus limiting selection to a group much more likely to have functional significance. The ability to select both genes with relevance to cancer biology and the specific SNPs within those genes that cause deleterious protein changes combines the strength of the candidate approach (genes are selected because of a priori interest) with the power of chip-based approaches to study thousands of candidates. Accordingly, Rudd and colleagues find associations with genes that are highly plausible *(ATM*, *BRCA2*, and *CHEK2)*.

The critical and complementary role of population studies can be appreciated by the *ATM* finding. *ATM* has a critical role in DNA repair, and its location near a commonly observed cytogenetic abnormality in CLL at 11q13 makes it a highly plausible candidate gene in CLL. Nevertheless, an earlier linkage study excluded the gene.[3] Since Rudd and colleagues observed estimated odds ratios in the 1.7 to 2.1 range, the signal would have "passed below the linkage radar"; that is, occurred below the limit of detection for linkage but comfortably within the range detectable in case-control design using large population samples.

Much remains to be done. Future linkage studies should involve consortia that combine precious high-risk CLL kindreds to provide the best chance to detect signals from the so-far elusive high-penetrance genes. It is axiomatic that the specific candidates implicated require independent validation in well-designed studies from diverse populations. In spite of the reasonable sample size and conservative threshold for significance, the general concern for false positives mandates a very cautious view of the specific candidates identified. Since the authors selected both the genes and the SNPs, it is likely that many possible pathways and genes are under- or unrepresented, so alternate whole-genome approaches (eg, the indirect approach) that represent the whole genome in an anonymous but more balanced manner will be eagerly awaited. Chip-based approaches like that of Rudd and colleagues should see broader application as investigators move to apply advanced genomic approaches to the problem of genetic susceptibility in common hematologic malignancy. ■

REFERENCES

1. Goldin LP, Ishibe N, Sgambati M, et al. A genome scan of 18 families with chronic lymphocytic leukemia. Br J Haematol. 2003;121:866-873.
2. Sellick GS, Webb EL, Allinson R, et al. A high-density SNP genomewide linkage scan for chronic lymphocytic leukemia-susceptibility loci. Am J Hum Genet. 2005;77;420-429.
3. Bevan S, Catovsky D, Marossy A, et al. Linkage analysis for ATM in familial B cell chronic lymphocytic leukemia. Leukemia. 1999;13:1497-1500.

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Comment on Chen et al, page 669

Alert and sniffy-nosed about *Mll-AF4*?

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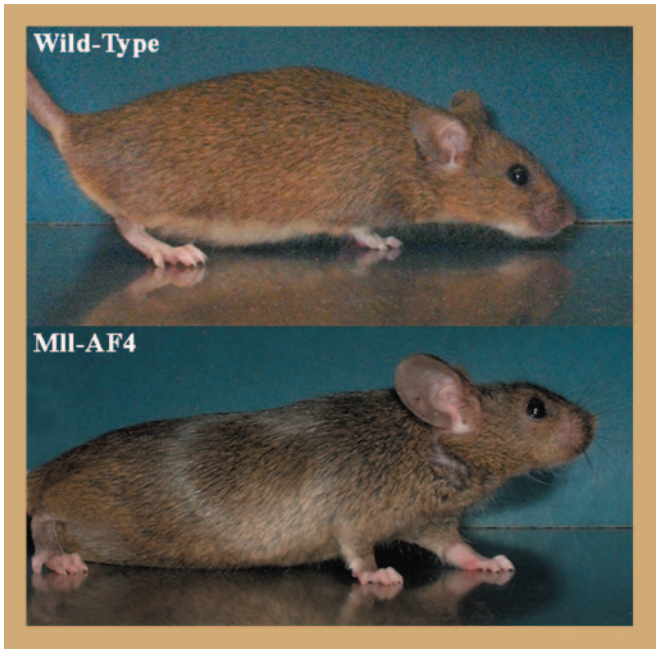
A report from the Kersey laboratory documents the long-awaited successful establishment of a murine model for leukemogenesis by the *Mll-AF4* fusion gene.

The *MLL* gene is notorious for its promiscuous fusion with multiple alternative partner genes in leukemia, and much ingenuity has been expended in attempting to identify shared or common functional attributes of the resultant proteins.[1] Several other intriguing biologic questions arise from a consideration of these different MLL chimeras, including the basis of selective myeloid or lymphoid lineage phenotypes. In principle, this might be ascribed to cell context–dependent transformation or, alternatively, the imposition of a particular lineage phenotype on progeny of multilineage progenitor or stem cells.

Several models for transformation and leukemogenesis by *MLL* fusion genes have been reported,[2] including transformation assays (by replicate cloning) in vitro and leukemogenesis by transformation of stem/progenitor cells in vitro followed by transplantation in vivo, MLL fusion knock-ins (via embryonic stem [ES] cells), or by engineering translocations.[3] What has been missing from this story to date (though many labs have tried and failed) is any model at all, in vitro or in vivo, for *Mll-AF4*, the predominant fusion gene of infant acute lymphoblastic leukemia (ALL) where it is associated with a pro-B or pro-B/monocyte phenotype, remarkably short latency, high white-cell counts, and high-grade malignancy.[4]

Chen and colleagues report in this issue of *Blood* a model based on *Mll-AF4* knock-in to ES cells that has an intriguing phenotype supporting, so the authors contend, an "instructional" model of lineage affiliation (Metzler et al[5] are reporting a very similar phenotype with *Mll-AF4*). In addition to a general skeletal/facial phenotype (big ears, stubby nose; see the figure), young *Mll-AF4* mice have a striking increase in both pro–B cells and mixed lympho(B)/myeloid cell colonies with replicate cloning ability (ie, they are transformed). This phenotype is also observed, though to a lesser extent, in *Mll-AF9* mice. Malignancies occur but only after a very protracted latency (200-700 days; median, 17 months) and then with a predominant follicular B-lymphoma phenotype. This phenotype was not seen with *Mll-AF9* mice; they develop myeloid leukemias, again with long latencies. The early, transformed, or preleukemic stages of disease in these mice more or less mimics what could be expected from the corresponding human disease if, as assumed, the equivalent target cell has been hit. But what is clearly different in this new model is the absence of pro–B-ALL with a brief latency as seen in patients. The authors' speculation that the critical secondary mutations involved in infant ALL are missing seems very plausible, but this still begs an interesting question: why do human fetal stem cells/progenitor cells transformed by *Mll-AF4* so rapidly acquire necessary secondary genetic events in the pro–B-cell compartment? One possibility is that transformed pro–B cells in the human fetus are exposed to the transplacental mutagenic chemicals that induced the *Mll-AF4* fusion itself,[6] and indeed may be ultrasensitive to such insults.[7] In the current mouse models, and in the absence of



Wild-type (top) and *Mll-AF4* (bottom) mice at the age of 5 weeks. *Mll-AF4* mice have a shortened face and large ears. *Mll-AF9* mice have a more pointed face and large ears. See the complete figure in the article beginning on page 669.